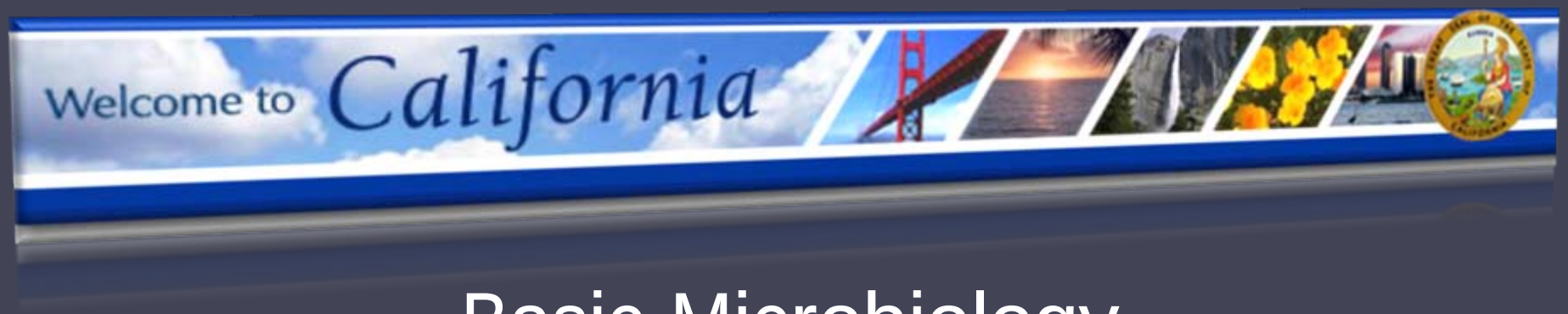

Basic Microbiology

Basics of Infection Prevention
2-Day Mini-Course
October-November 2011

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Objectives

- Describe role of the microbiology lab in infection prevention
- Describe factors that can adversely affect reliable micro results
- Review definitions
- Discuss the role of the gram stain
- Review common pathogens for HAI
- Review laboratory markers for hepatitis viruses

Microbiology and Infection Prevention

Microbiology laboratory has two important functions in infection prevention

- **Clinical**: diagnosis and management of infections
- **Epidemiological**: understand infectious microbes in patients (and populations), to find sources and routes of transmission necessary for prevention efforts

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Clinical Microbiology

Physician's perspective:

- What's growing?
- What antibiotic can be used?
- Determined either by predictive value of the organism type (e.g. gram negative bacillus) or by complete result with sensitivities

IP or Epidemiologist's perspective:

- Surveillance
- Need both the organism's genus and species (e.g. *Pseudomonas aeruginosa*) and sensitivity pattern
- For determining clusters/outbreaks and assessing trends

Berg's Rule of Thumb #1

No lab test is 100% accurate 100% of the time

Interpret all results accordingly !!

Assessing Accuracy of Lab Results

Many factors can affect accuracy of laboratory tests

1. Pre-analysis:
   - How was specimen collected, handled, transported, preserved prior to arrival in the lab?
2. Analysis:
   - Were correct agar plates used? Incubated at correct temp? Skill of the micro tech? Accuracy of biochemicals and instrument system?
3. Post Analysis:
   - Accurate result transcription in computer systems? Did results get communicated to the doctor accurately?

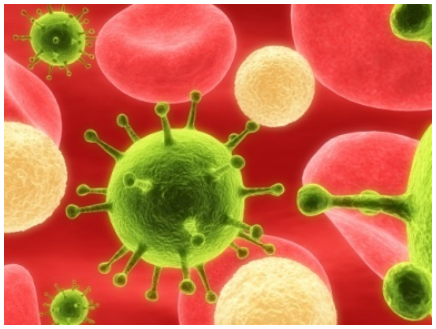
Robert Berg, 2009, Micro for ICPs.
www.apicsierra.org/page6.html

Berg's Rule of Thumb #2

Just because a bug is growing does not mean it's causing disease…Colonization??

- For normally sterile body sites, growth may indeed be an infection
- Interpret all cultures knowing what pathogens would typically/normally grow in that body site

Definitions



- WBC = white blood cells = leukocytes

Major types of WBC

- PMN, Polys = polymorphonuclear leukocytes
  - If increased, likely a bacterial infection
- Segs, Neuts = segmented neutrophils
- Lymphs /mononuclears = lymphocytes
  - If increased, can presume a viral infection

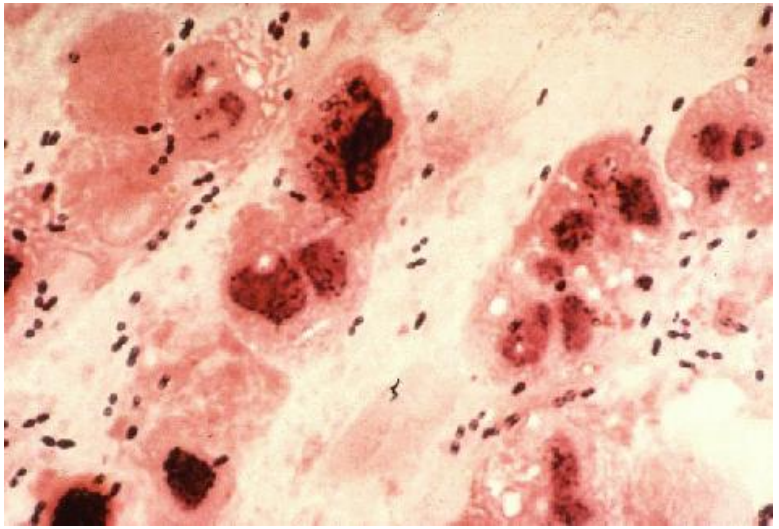
Gram Stains

- Helpful in guiding initial empiric therapy
- Helpful in evaluating quality of culture result
- Does not improve patient outcome if the results don't get to the physician ASAP

Sputum Gram Stain

Will see

- Squamous epithelial cells (SEC)
- WBC
- Bacteria

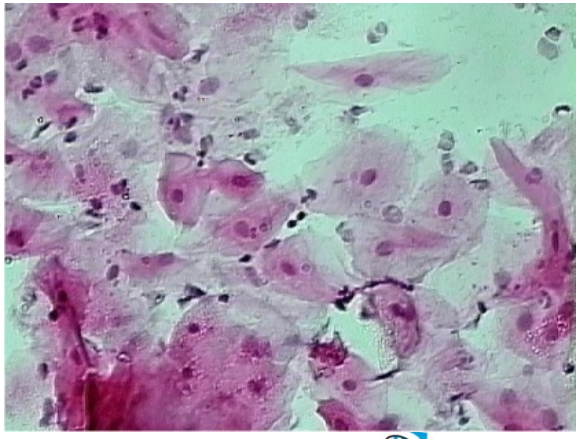


The presence of SEC is indicative of contamination

Sputum Gram Stain – 2

SEC (squamous epithelial cells, under low power field)

- <10 excellent specimen, no appreciable oral contamination
- 10-25 equivocal specimen, but acceptable
- >25 reject due to unacceptable levels of oral contamination



Sputum Gram Stain – 3

WBC (under low power field)

- <10 no infection
  (or not much of a response due to immuno-suppression, PCP, Mycoplasma, viral, etc)
- 10-25 equivocal
- >25 infection is evident (purulent)

Lower Respiratory Cultures

- Sputum and bronchial wash: often contaminated with oral flora
- Protected brush specimen: not contaminated with oral flora
  - semi-quantitative method recommended
  - put brush into 1.0mL TSI broth; vortex; inoculate agar with urine loop
  - reported as number of CFU/ml*
- Tracheal aspirates: often shows colonizers

*CFU = colony forming units; ml = milliliter

Common Lower Respiratory Tract Pathogens

- *S. pneumoniae*
  - primarily community acquired pneumonia (CAP)
  - uncommon as healthcare-acquired pneumonia
  - aminoglycosides can select for S. pneumo
- *H. influenzae*
  - primarily CAP
- *Moraxella (Branhamella) catarhallis*
  - most often CAP, but can be hospital acquired
- *S. aureus*
  - CAP and hosp acquired
  - ⇑ mortality; must be recognized quickly

Common Lower Respiratory Tract Pathogens - 2

- *Pseudomona aeruginosa*
  - often ventilator- or ICU-related
- Mycoplasma
  - CAP
- *Stenotrophomonas maltophilia*
  - ventilator- or ICU-related
- Yeast
  - not usually infecting organism (pneumonia or LRI) unless constitutes ≥70% of all organisms present in specimen and oral contamination can be ruled out

Cerebrospinal Fluid (CSF) Pathogens

- Source: often upper respiratory flora
- Meningitis due to gram negative rods or Staphylococcus usually with predisposing factors such as trauma
- Adult, most common: Strep pneumo (gram positive cocci in pairs)
  - generates increased WBC response
- Meningococcemia: Gram stain showing gram-negative diplococci is diagnostic
  - A true infection emergency
-

Meningitis

Onset of Symptoms

↓

Patient presents to MD for medical evaluation

Lumbar Puncture (LP)

Bacterial	Viral
CSF Cloudy	CSF Clear
Elevated Protein	Normal or Elevated Protein
Decreased Glucose	Normal Glucose
WBC; Positive Neutrophils	No organisms on gram stain
Organisms on gram stain	

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Blood Cultures

- A single blood culture consists of two bottles
  - Bottles designed to recover aerobes and anaerobes
  - Irrelevant which bottle has growth or if both or only one bottle has growth
- Adults: low numbers of bacteria in blood (≤30/mL)
  - can lead to negative gram stain and false negative
  - require relatively large volume for blood culture
  - Less blood needed for children due to larger number of bacteria per cc of blood

Polymicrobic blood culture: if intra-abdominal enteric event

- e.g. ruptured appendix, bowel surgery, intestinal perforation

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Blood Culture Contaminants

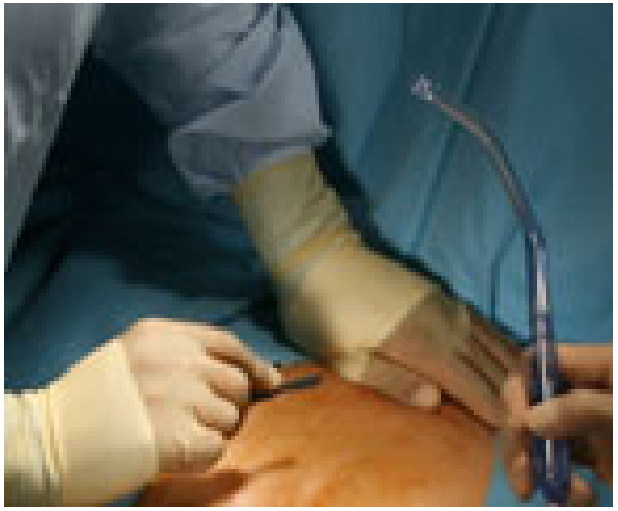
Common contaminants

- Coag neg staph
- Diphtheroids
- Bacillus
- Proprionibacteria
- Viridans strep
- Aerococcus
- Micrococcus

For these bacteria to be interpreted as causing infection, two sets of blood cultures are required PLUS specific signs and symptoms (fever,

Common Pathogens of Superficial Surgical Site Infections (SSI)

- Not usually anaerobes
- Generally skin flora, but not necessarily
- Can be gram negative rods (GNR)



Common Pathogens of Deep and Organ Space SSI

Caused by anaerobes and aerobes

- Anaerobic examples
  - *B. fragilis*
  - *Clostridium*
  - *Peptostreptococcus*
  - *Propionibacterium* (septic arthritis, endocarditis, suture sites for craniotomy)
- Aerobic examples
  - Staphylococcus
  - Streptococcus
  - GNRs

Common UTI Pathogens

- Gram negatives
  - *E. coli*: Cause 80% of all UTI
  - Proteus, Klebsiella, Enterobacter, Pseudomonas, Gardnerella, 5-10%
- Gram positives
  - MRSA, Enterococcus, *Staph saprophyticus,* 10-20%
- Positive leukocyte esterase and/or nitrite can be helpful in determining infection status
- Increased WBC with negative cultures may indicate chlamydia or gonorrhea.

Extended Spectrum Beta-lactamase (ESBL)-producing Gram-negative Bacteria

- **Cephalosporins:** developed to combat emergence of β-Lactamase producing GNR
- **Soon there was Resistance:** to 3rd generation Cephalosporins (eg: cefotaxime, ceftazidime, ceftriaxone) and Monobactams (e.g.: aztreonam)
- **ESBL still sensitive to:** Cephamycins (cefoxitin, cefotetan, cefmetazole) and carbapenems (e.g.: meropenem, imipenem)

ESBL (continued)

Drug of choice for treatment of ESBL infections:

- **Carbapenems** (mero, dori, imi, erta)
  - Carbapenemase breaks down all Penicillins, Cephalosporins, Carbapenems
  - Carbapenems: “the last resort for gram negative infections”
  - Most potent ß-lactam class against almost all Enterobacteriaceae

Unfortunataly, Carbapenemase-resistant Enterobacteriaceae (CRE) beginning to emerge, leaving few treatment options *(scary)*

Common Bowel Flora

- Normal mix of bacterial flora keeps numbers of yeast, *C. difficile*, and other potential pathogens in the gut in check
- With altered flora:
  - yeast can proliferate
  - *C. diff* can proliferate
  - pseudomonas can proliferate
  - VRE can proliferate
  - Etc, etc, etc

Hepatitis **A** Viral Markers

Hepatitis A Virus (HAV)

- HAV, total – current or past HAV
- HAV, IgM – definitive diagnosis of active HAV infection

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health.

Acute hepatitis A requires immediate notification.

Hepatitis **B** Viral Markers

Especially important in women of chidbearing years

Hepatitis B Virus (HBV)

- HbsAg – current or chronic HBV
- HbsAb – recovery or immunity to HBV
- Anti-Hbc – current or previous HBV infection
- Anti-Hbc IgM – recent acute infection
  - If also HbsAg ⊕ - acute infection
  - Distinguishes acute from chronic infection
- HbeAG – highly infectious
  - Guidelines exist for HCWs who are HbeAG positive

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health

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Interpretation of the Hepatitis B Panel		
Tests	**Results**	**Interpretation**
HBsAg	negative	Susceptible
anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	Immune due to natural infection
anti-HBc	positive	
anti-HBs	positive	
HBsAg	negative	Immune due to hepatitis B vaccination**
anti-HBc	negative	
anti-HBs	positive	
HBsAg	positive	Acutely infected
anti-HBc	positive	
IgM anti-HBc	positive	
anti-HBs	negative	
HBsAg	positive	Chronically infected
anti-HBc	positive	
IgM anti-HBc	negative	
anti-HBs	negative	
HbeAG	positive	Highly infectious

Hepatitis **C** Viral Markers

Hepatitis C Virus (HCV)

- Anti-HCV
  - Presence of antibodies to the virus, indicating exposure to HCV
  - Active vs. Chronic vs. Resolved - ?
- HCV RIBA
  - Confirmatory test of antibodies to the virus
  - Demonstrates if HCV was true positive (present or past is unanswered)

All Hepatitis (acute and chronic) are reportable communicable diseases via local public health

Role of Microbiology in HAI Prevention

Critical to

- Outbreak management
- Performing additional tests for epidemiologic analyses
- Infection surveillance
- Knowledge of new microbes or unusual resistance
- Design of antibiotic formulary (antibiogram)
- Interpretation of microbiological results
- Education of health care staff

Questions?

For more information, please contact any HAI Liaison Team member.

Thank you